A STUDY OF THE MICROSCOPIC PHENOMENA OF INFLAMATION, WITH SPECIAL REFERENCE TO DIAPEDESIS OF THE WHITE BLOOD CORPUSCLE.

By Charles F. Craig, M. D.

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Away back, in the olden times, observers noticed that a majority of the diseases of mankind were accompanied by certain symptoms, and these were constant and formed an unbroken chain in many instances. These symptoms were: swelling, redness, pain and heat in the diseased part or organ, and also, at times, an increase in the pulse rate and a rise in the general temperature of the body.

We, to-day, have no difficulty in recognizing here the characteristics of inflammation and fever. The tissue which was red, swollen and painful was said to be inflamed, while if there were an increased pulse rate, higher temperature, and a change in the chemical activities going on within the body, there was said to be fever.

The connection between the two was generally easily seen, but sometimes one occurred without the visible presence of the other, and their connection became obscured, but there came a day when the microscope was introduced into medical research and then the production of inflammation and fever were closely studied and light was shed on what was before darkness.

By careful experiment the following facts have been proved regarding the origin of inflammation. First, there is, on irritating the tissue experimented upon, a contraction, followed by a dilation of the capillaries, then a migration through the capillary walls of leucocytes or white blood corpuscles, which spread into, and increase in the surrounding tissue; then a blood stasis in the dilated capillaries and a congestion in the inflamed area.

These are accompanied by disturbance in the nutritive processes and a heightened temperature.

With the thought in my mind that we learn new things only by more carefully and patiently observing old ones, I spent nearly all of the three months in the Summer of '93 in studying beneath the microscope the following phenomena of commencing inflammation: dilatation of the capillaries, slowing of the blood stream, and the escape of the leucocytes or white blood corpuscles from the blood vessels into the surrounding tissue.

I made these observations with but little hope of discovering any new facts where such observers as Cohnheim, Lister, Stricker, Böttcher and others had so carefully and scientifically worked but simply to convince my own mind of the truth of their revelations, and to decide, if possible, certain points regarding the diapedesis of the leucocytes which are still in dispute.

It will hardly be necessary for me to state that my results confirmed their observations, and in one or two points, my observations, although differing somewhat from theirs, have cleared up to my own mind, disputed questions, the principal of which is that regarding the part taken by the leucocyte in its passage through the vessel wall.

I do not wish to put forward any new theory, but simply state that which I observed, leaving to others the task of working out the theory which will accord with the facts. My observations were made upon the foot-webs and mesenteries of ten frogs and in this paper I do not intend to give a description of each individual experiment which would be but repetition, but simply to state my general results and conclusions.

The drawings inserted were made free hand, without a camera lucida, which will account for their crudity and comparative simplicity.

METHOD.

The method I have followed is simple in the extreme.

First, I constructed a platform for the stage of my microscope, on which to place the frog. This consisted of the parts which the diagram on the opposite page shows.

A is a piece of cork, twelve inches long, four inches wide and half an inch in thickness. At the center of its long diameter and

an inch from its side I cut a hole about two inches long and an inch wide, as at B. Over this I placed a glass slide, such as is used in microscopy, sinking its two ends, (a and b in cut) even with the surface of the cork. As will be seen the slide entirely covers the hole in the cork, which reaches from c to d. The circle drawn in the center of the slide at f merely indicates the situation of the aperture in the stage of the microscope, through which the light comes when the apparatus is placed upon it.

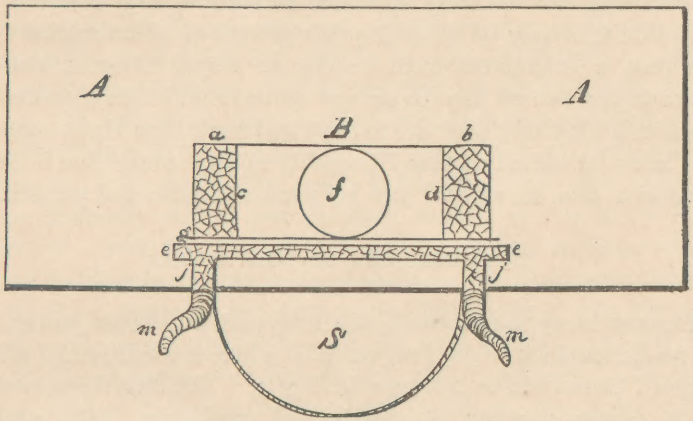

FIG. 1.

A quarter of an inch from the outer edge of the slide (at g) I cut a groove in the cork running a little beyond the ends of the slide and parallel with it, and a quarter of an inch from each end of this groove, another groove at right angles to it, which is connected with a rubber pipe (m) to allow for drainage, if needed. The cut is drawn as the apparatus looks when it lies upon the stage of the microscope, the posterior portion of which is seen at S. This apparatus is simply fastened upon the stage of the microscope, seeing that the glass slide covers the aperture in the stage, and the frog arranged upon it, after being curarized by injecting a little of a watery solution of the poison in the dorsal lymph sac.

If the web of the foot is to be examined it is simply stretched across the slide at "f" and held in place by felted pins, placed between the toes but not piercing them.

If the mesentery is to be examined, the frog is placed on the

apparatus after being curarized, the abdomen carefully opened and the intestine gently lifted out with felted forceps and placed in the groove "e" thus stretching the mesentery smoothly over the slide at "f," where it can be easily examined. My method was to get a fresh frog from a pond near by, each time I needed one.

On the first ten frogs I made no observations whatever of the mesenteries, but only of the foot webs, while on the last ten I observed the changes in the mesenteries only. I tried to observe as carefully and as long at a time as I could, and I would say here that it is only by watching this process of inflammation from the time it commences until we can no longer follow it with our microscopes, can we hope to see and understand all its phenomena. I found it intensely tiresome to observe longer than eight consecutive hours at a time so that I probably missed much that I might otherwise have seen had I had greater endurance and patience.

EXAMINATIONS OF FOOT-WEBS.

In examining the foot-web of a frog with a medium power, say a two-thirds inch objective, we will notice a condition of affairs which I have tried to illustrate in Fig. 1. The first thing noticed is the great number of vessels and capillaries, filled with a swiftly moving current of blood; so swiftly indeed does it move, that the constituents of the blood can not be distinguished, and we see it only as a never ending motion, without known composition.

At various situations in the field beautifully branched pigment cells are seen (d Fig. 2), and on carefully focusing we can distinguish the cells of the surface epithelium, each cell containing a barely distinguishable nucleus (e, Fig. 2). This is the appearance of the web before it is acted upon by an irritant, and such will be the appearance for hours, if the part is kept moistened and not irritated.

But on touching the web lightly with nitrate of silver, or even scratching it with a needle, we get an entirely different appearance, and the process of inflammation begins. At once there is a dilatation of the arteries, this dilatation extending gradually to the veins and capillaries. As far as I could determine this dilatation was not preceded by any contraction, and affected the arteries

mostly, and the capillaries but slightly, although the ratio may have been alike. This dilatation seemed to increase steadily for about six hours, although Cohnheim states that it steadily and slowly increases for twelve hours.

At the beginning of this dilatation there was an acceleration in the flow of blood plainly noticeable, but this, after lasting only about an hour, resulted in a considerable retardation of the flow, the vessels still remaining dilated. This is shown in Fig. 3.

After an hour, and sometimes in the smallest capillaries, much sooner, pulsation became evident and the current of blood so slow that, in the small veins and capillaries, the individual corpuscles became distinguishable. They can first be distinguished in the veins. In two of my observations, this dilatation took place and subsided without any of the other phenomena of inflammation occurring, but, afterwards, a second dilatation with slowed stream came on slowly, which was constant, lasting as long as the caues operated. This last dilatation was, no doubt, *the* vascular change of the inflammation, due to the irritation. Returning to the time when the corpuscles are first to be distinguished, the following phenomena were observed. Using now a one-sixth objective. As the blood stream became slower, white corpuscles or leucocytes were seen in the plasmatic layer in the smaller veins, rolling along sticking here and there, coming to a stand still for a few seconds, then rolling on again, and at last often sticking fast to the vessel wall, resisting all attempts of the current to dislodge them. A vessel at this time presents the appearance illustrated in Fig. 3 and 4, the white corpuscles being scattered here and there along the vessel wall, while the central canal of the vessel, enclosed on each side by the plasmatic layer in which the leucocytes lie is filled with the red corpuscles which can be distinguished very readily as the stream gets slower and slower. One of two things now generally occurred, either the leucocytes continued to accumulate on the vessel wall or else the vessel became so thickly packed with corpuscles as to present the appearance of a red injection mass, all movement ceasing. (See Fig. 5.) The contents of the vessel sway forwards and backwards with the impulse transmitted to the stream by the beating of the heart. This is known as the stage of oscillation and is succeeded by a complete stagnation, called stasis, in which no movement of any kind oc-

curs. I have spoken of the accumulation of leucocytes along the vessel wall, as though in this last instance it did not occur; by this I mean that in my observations the vessel filled so rapidly that the accumulation of leucocytes was hidden from view, although if the field thus obscured be watched long enough the leucocytes are found congregating outside the vessel, thus showing that diapedesis still goes on. Where, however, the blood stream still continues to move on, the leucocytes accumulate more and more, until the vessel wall in some cases, becomes lined with them often two or three cells in thickness, as in Fig. 4. If such a small vein be watched very carefully for a varying space of time, from half an hour to several hours the following phenomena are seen to take place. (It is not necessary, however, that the vessel wall be lined with leucocytes in order that these phenomena take place, for in all those observations in which I obtained the clearest and best view of the process the leucocytes were only situated here and there upon the vessel wall.) As the vessel wall is watched, the leucocytes are seen to gradually penetrate its wall, and finally to emerge upon the other side, having passed entirely through the vessel wall into the surrounding tissues. This process seems to be divided into four distinct acts which I have tried to roughly illustrate by Figs. 3 to 9. At first the leucocyte is seen to adhere to the vessel wall, as in Fig. 3 and 4, then it sends a process into and through the wall, as in Fig 6, following this, the remainder of the cell is drawn after or follows this process, at one time in its passage appearing, constricted in the middle in Fig. 7 and finally, the leucocyte passes completely through the wall, and moves into the surrounding tissue, by virtue of its amoeboid movements.

In these four acts, the process, known as the diapedesis of the white blood corpuscles or leucocytes consists and it is said to be due partly to the independent motility of the leucocytes themselves and partly a filtration of the colloid mass of the cell by the force of the blood pressure and probably taking place through the spaces between the endothelial cells, the vessel wall itself having been altered in some way, by the process of inflammation.

In my observations, I very carefully watched all of the phenomena and could observe nothing peculiar during the first stage, *i. e.*, adherence to vessel wall, but, in the second stage,

i. e., when the cell sends out its process into the wall, I observed a strange thing, namely : that the process sent into the wall by one cell was almost identical with the process that any other cell sent out both in size and general outline.

This process was a cone shaped rather pointed one, which seemed to work its way into the wall of the vessel, and in fact, it seemed to quietly and steadily push its way on and in, until at last it emerged upon the other side of the vessel. I also observed in every case which I observed, where, after adhering the leucocyte was swept from its hold and carried on in the current, that it retained its penetrating process, (see Fig. 9 a and b) and did not as long as I could see it, regain its irregularly circular shape.

From these two facts it seems more than probable that this penetrating process is due to the action of the cell itself rather than that it is such a one as would be produced by the blood pressure acting upon the soft colloid material of the cell and pressing it into the vascular wall, for a process so formed would disappear as soon as the leucocyte was swept off into the current.

Again, the uniformity of the shape of the process tends to favor the view that the leucocyte penetrates the vessel wall mostly by its own independent power of locomotion.

Now. as the leucocytes penetrate the vessel in greater and greater numbers, as serum accumulates also, the surrounding tissues become infiltrated as in (Fig. 9) and the field observed through the microscope becomes covered by a false membrane composed of leucocytes, connective tissue cells, film and serum. The above facts constitute the phenomena of inflammation which I observed in the web of the frog's foot ; I did not carry my observations further, as I mostly wanted to study the diapedesis of leucocytes, and I found that owing to the amount of material in the field, other than blood vessels, *i. e.*, pigment cells, epithelial cells, etc., I could not obtain a good clear view of the process, and I accordingly conducted all my other observations upon the mesenteries of frogs, and obtained, I believe a much more accurate view of every step of the process as far as a microscope will reveal it. From the foot-web of a frog then I observed besides the process usually described as the adherence to and penetration through the vessel wall of the leucocyte two distinct and characteristic phenomena.

1. The process, which any leucocyte sends into the vessel wall in penetrating it, is almost identical in size and shape, with that which any other leucocyte sent off in the same act, or in other words, the leucocytes all send off similar processes into the wall of the vessel before penetrating it. 2. In all the cases which I observed, if a leucocyte, after sending off such a process, is swept from the vessel wall by the current it always retains that process and does not return to its former shape, providing it had really began penetrating the wall, as long as it remain in the field of vision.

OBSERVATIONS ON THE MESENTERIES.

At this point I transferred my observations to the mesenteries of frogs, but before doing so I made a thorough examination of the corpuscles of the frog's blood with the following results: Besides the oval red corpuscles I found there are two distinct kinds of white corpuscles present in the frog's blood. See Fig. 10.

1. The most numerous are large, about $\frac{1}{1200}$ in. in diameter, irregular in outline, having fine projections from their surface and finely granular. They have two, or generally, three nuclei or three part nucleus.

2. The second variety is less numerous, more coarsely granular, smaller and contain but one nucleus. The granules in the protoplasm are sometimes seen to rush from one side of the corpuscle to the other. They both have active amoeboid movement. Unfortunately I could not distinguish these varieties in my observations, on the process of inflammation for the following reasons:

1. In the small vessels, which I preferably watched, the white corpuscles were so few in number that the two varieties did not happen, in the cases I observed, to occur together, while if the leucocytes were present in larger numbers, they were so crowded that the difference could not be distinguished.

2. The nuclei of the leucocytes cannot be readily distinguished without using reagents, which would have interfered with the process of inflammation.

I found it necessary, as I have stated, in order to clearly define the neuclei to use reagents. Adding to the blood a little dilute acetic acid, clears up the surrounding protoplasm and brings

the nuclei very clearly into view. Magenta may now be added which gives a very fine stain. Adding dilute alcohol will also bring the nuclei into view.

Having now thoroughly examined the blood, I next examined my field of operation, the mesentery. This is composed of the following elements, being a most typical example of all serous membranes. It is transparent and consists of:

1. Very delicate connective tissue fibres.
2. Connective tissue cells.
3. Some elastic fibres, the whole being traversed by a beautiful net-work of lympatics and blood vessels. In order to watch for a long time, the process of inflammation in the mesentery, it is necessary to keep it moistened by a weak salt solution (¾ per cent.)

Carefully opening the frog's abdomen and spreading the mesentery over the slide as described, I observed the following phenomena:

A slight inflammation is caused by simply exposing the mesentery to the air and there is a slight dilatation of the arteries with an accelerated current, and we get an appearance like that in Fig. 11.

The stream of blood is so rapid that it is impossible to distinguish the individual corpuscles, but gradually, the irritation being kept up, in from four to five hours, if in a medium sized vein the stream begins to grow slower, and soon the corpuscles become distinguishable, first in the smaller vessels, (see Fig. 12).

This slowing occurred sooner in the mesentery than in the web of the foot, and more rapidly the greater the irritation.

As the stream grows slower the same phenomena are observed as in the web, *i. e.*, the red corpuscles occupy a central position in the vessel said to be due to their greater specific gravity, for in 1868, Schklarewsky showed by physical experiment that particles of least specific gravity in glass capillaries are pressed toward the capillary wall, while the ones of greater specific gravity remain in the center of the stream.

On either side of the stream of red corpuscles is a zone of plasma in which most, but not all, of the white corpuscles are moving. In the smallest capillaries the red corpuscles move along in single file, sometimes being compressed between the walls,

but regaining their former shape by virtue of their elasticity on reaching larger vessels. The white corpuscles are plainly seen rolling along, sticking here and there on the vessel wall, sometimes being swept off again by the axial current. There is now an appearance like that sketched in Fig. 12 the red corpuscles being plainly distinguishable, and the white ones adhering here and there to the vessel wall.

At this point the leucocytes begin to penetrate the vascular wall, and as I carefully watched the process, in nearly a hundred different locations in the mesenteries, from nearly all of them I obtained the following results.

1. After a leucocyte has remained for a variable length of time upon the vessel wall, and just before it can be seen to send a process into the wall, the granular matter in its protoplasm all seems to be in motion and congregates thickly at that portion of the leucocyte furthest from the vessel wall, or, in other words, nearest the axial stream of the vessel. (See Fig. 13).

2. The leucocyte next sends off a process which was almost exactly the same in outline and size, in every case which I observed, being composed of protoplasm entirely free from granules, and which seems to gradually push or work it's way into the wall. See Fig. 13.

3. Now occurs a most singular thing. As the vessel wall at last gives way and the process of the leucocyte emerges upon the outer side of the vessel, the granules, which have, during the penetration of the wall, been congregated at the point in the leucocyte furthest from the wall, these granules fly swiftly through the passage thus made in the vessel wall by the protoplasm of the cell, from the interior of the vessel to the furthest point of the process outside the wall, leaving the part of the leucocyte not yet through the wall, free of granular matter. This now passes through and the leucocyte regains its former shape, the granules becoming distributed over its surface. See Figs. 14 and 15.

In several instances while the leucocytes were in the act of penetrating the vessel wall, they were swept from it by the blood current but the granules still held their position in the part of the cell away from the penetrating process, and more than this, in one case, a leucocyte so swept away adhered again to the wall while still in the field and penetrated it with the self same process,

passing through all the stages mentioned. These phenomena indicate to my mind that the leucocytes penetrate the vessel wall largely by their own activity, and I am convinced that such is the fact.

As the blood-stream becomes slower and slower the process becomes obscured by the accumulation of leucocytes, and when at last the current stops, the vessel becomes filled with them and diapedesis can no longer be watched.

To sum up, then, the following facts are those which I think are most important, and which I have not as yet seen any other account of:

1. The leucocytes, in penetrating a vessel wall, send off processes similar in shape and size.

2. If a leucocyte, while in the act of penetrating the vessel wall, be swept off into the blood current, it retained its penetrating process, as long as it remained in the field.

3. The granular matter in the leucocytes becomes massed in the portion of the cell furthest from the penetrating process, so that the process contains only clear protoplasm.

4. If the leucocyte be swept away while penetrating, the granules still retain their position, and in one case, the leucocyte was seen to again attach itself to the wall, and use the same process in penetrating it.

5. The clear protoplasmic process works its way through the wall and in the channel of protoplasm thus formed, the granular matter rushes through the wall and occupies the clear process which is now outside the vessel, leaving clear the portion of the leucocyte still within the vessel.

6. This clear portion soon comes through the vessel wall, and the leucocyte regains its original shape, the granules being distributed over its surface. (See Fig. 16.)

The thought came to me that perhaps it was the coarsely granular corpuscles, heretofore described, in which the granules are seen to change their position, that were the ones which penetrated the wall, but when I considered the small number of this variety as compared with the large number of leucocytes so escaping, I am forced to conclude that it is probably the finely granular variety which constitute the larger part of the escaped leucocytes. Of course the phenomena described give rise to many interesting

questions, but to me it seems evident that the condition brought about by irritation, causes in the leucocyte a certain abnormal activity which is manifested by these phenomena, and when we know that even in health some leucocytes escape from the vessels, it looks as if this was simply an exaggeration of a normal process. I could not determine as to whether the escape took place through the stomata or not.

In conclusion I would say that while some of the phenomena described are exceptional to hitherto made observations, still, in the words of Claude Bernard, "In physiological (or other) studies we must always carefully note any fact that does not accord with received ideas, for it is always from the examination and discussion of this fact that a discovery will be made, if there is one to be made."

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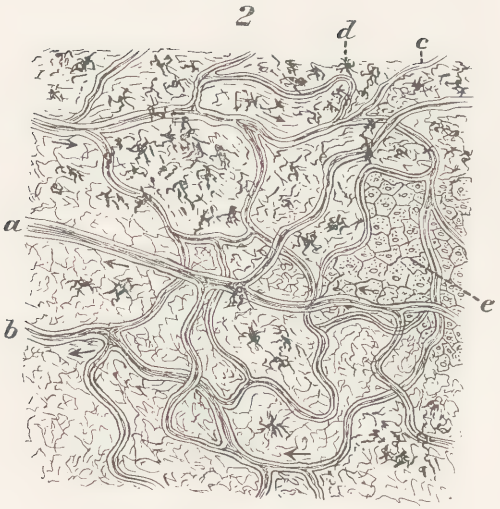
PLATE I.



Observations on Foot-web

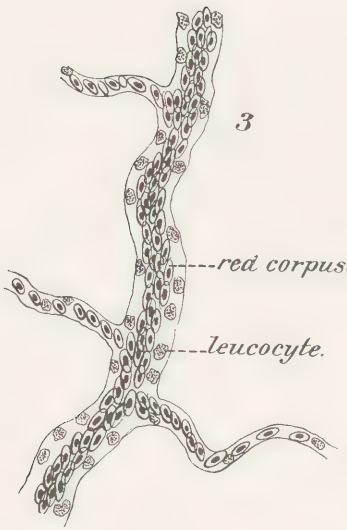


PLATE I.

Fig. 2. The web of a frog's foot as seen under a medium power.

a, an artery.

c, a capillary.

d, pigment cells.

e, surface epithelium, which can be seen by focussing.

The stream is so swift that the individual corpuscles cannot be seen.

Fig. 3. A vein, as it appears when the current begins to slow. The red corpuscles can be seen in the central portion of vessel, while the white corpuscles or leucocytes creep slowly along vessel wall, sticking here and there.

Fig. 4. Showing a vessel in which the current has nearly come to a standstill, and the leucocytes are seen arranged in rows along its wall.

PLATE II.

Fig. 5. A vessel in which the corpuscles have accumulated to such a degree that the current is blocked, and the vessel seems simply to contain an injection mass.

Fig. 6. Showing the leucocytes sending forth processes into the vessel wall, which resemble each other in shape and size.

Fig. 7. Leucocytes partly through vessel wall, showing constriction in their center.

Fig. 8. Leucocytes after penetrating vessel wall, showing their power of regaining their former shape.

Fig. 9. Appearance of a vessel and surrounding tissue after the process has gone on for some time.

At a and b leucocytes may be seen which have been swept from vessel wall, while penetrating it, showing how they retain the process.

OBSERVATIONS ON MESENTERIES.

Fig. 10. The two varieties of white corpuscle in the blood of the frog.

a, large corpuscles, having a bi-or tri-part nucleus, finely granular.

b, small corpuscle, having single nucleus and coarsely granular, and in which the granules may be seen to rush from one side to the other of the corpuscle.

Fig. 11. Appearance of mesentery of frog under a medium power, at time of exposure.

a, artery.

b, vein.

c, capillaries.

The current is so swift that the corpuscles cannot be distinguished. I have drawn the mesentery proper simply as a homogeneous mass.

PLATE II.

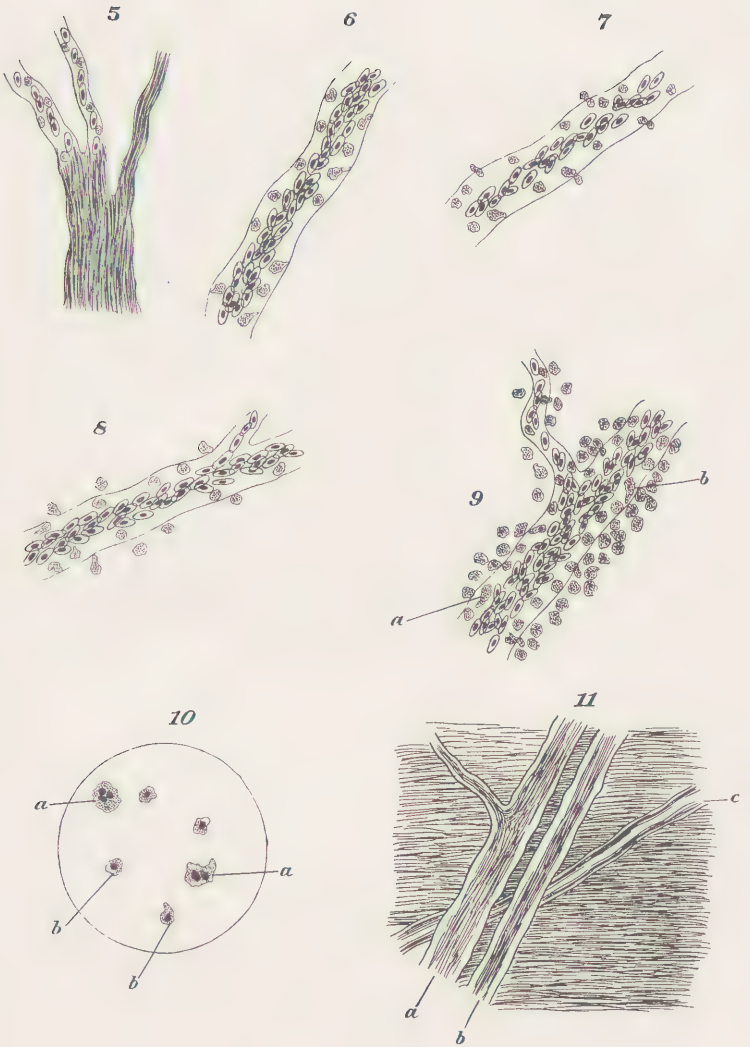


PLATE III.

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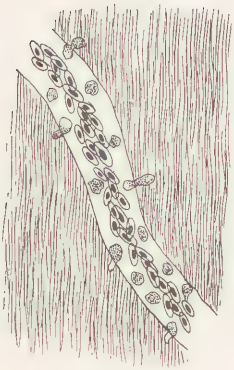

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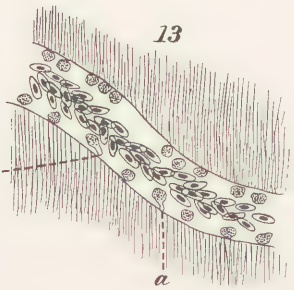

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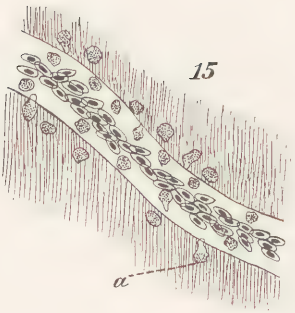

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Blood Capillary.

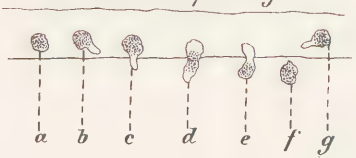

PLATE III.

Fig. 12. Stage of slowing of stream in mesentery.

a, an artery in which the current is still swift. This should have been shown as in Fig. 11.

b, a small vein, in which the current is slow, and the red and white corpuscles can be distinguished.

Fig. 13. Small vein of mesentery. Leucocytes may be seen sending off process, which is clear of granules. (a and b.)

Fig. 14. Small vein of mesentery. The leucocytes are seen partly through vessel wall, the penetrating part being clear, and some are seen in which the granules are flying through the passage made by clear protoplasm of the cell.

Fig. 15. Small vein of mesentery. Showing leucocytes which have penetrated and the granules are again distributed over entire cell, and also some which are nearly through wall and in which the granular matter is in the part which is entirely without the vessel wall, as at A.

Fig. 16. Diagram illustrating the points made.

a, leucocyte before penetrating.

b, leucocyte sending off process, and granules beginning to withdraw to farther end of cell.

c, lencocyte partly through wall, granules at upper end of cell.

d, granules flying through passage made by clear process.

e, granules arranged in portion of lencocyte farthest from vessel.

f, leucocyte after penetrating, resuming its original appearance.

g, leucocyte swept from wall showing retention of the clear penetrating process.